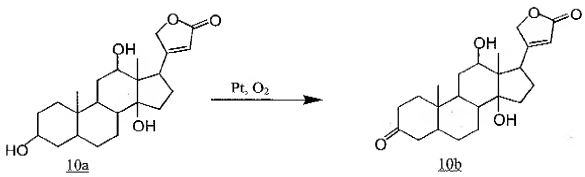
REPLACE pages 70-74 of the specification with the following pages:



**4. Preparation of Boc-Linked-Dig (10)**

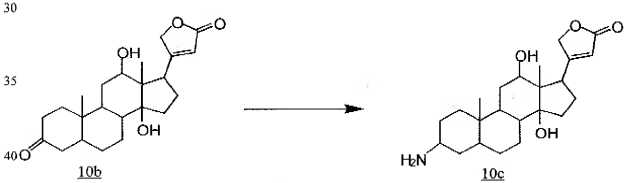
**a. 3-ketodigoxgenin (10B)**




10a 10b

Platinum oxide (489 mg) was suspended in 20 ml of deionized water and reduced to platinum metal in a Parr hydrogenation apparatus at 40 psi of hydrogen at room temperature for two hours. The platinum was then transferred to a solution of 500 mg (1.28 mmol) of digoxigenin **10a** ( prepared as described in Ferland, J.M. *Can. J. Chem.* 1974, 652) in 50 ml of dry acetone. The reaction was placed under a steady stream of air and stirred at room temperature for three days. The reaction was worked up by filtering the catalyst off through a pad of Celite and evaporating the solvent. 449 mg (90% yield) of the crude product **10a** was obtained, which was of sufficient purity for use in the subsequent step.

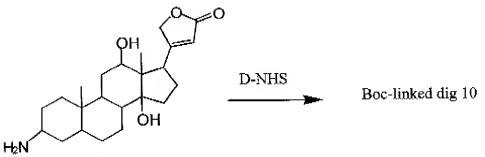
**b. 3-Aminodigoxigenin (10c)**



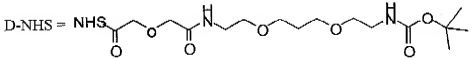

10b 10c

To 448 mg (1.26 mmol) of 3-ketodigoxigenin **10b** dissolved in 5 ml of methanol was added 100 mg (6.74 mmol) of ammonium acetate. The reaction was stirred at room temperature under argon for 30 minutes. Then 85 mg (1.35 mmol) of sodium cyano brohydride was added and stirring was continued for two more hours at room temperature. The 3-aminodigoxigenin **10C** was formed rapidly. The reaction was quenched by addition of 4 ml of glacial acetic acid and evaporated to dryness. The crude product was dissolved in 20 ml of n-butanol, then extracted with 25 ml of a solution of 1g/ml of potassium carbonate in water. The aqueous phase was washed with 3x25 ml of n-butanol. The combined organic phase was dried and evaporated to dryness to yield 365 mg (69%) of the **10c** as white solid. The crude product was of sufficient purity for use in the next step. NMR: ($CD_3OD$) 0.79(s,3H,C18), 0.96(s,3H,C19), 1.00(s, 3H, C19), 1.1-2.3 (m, CH2's and CH's), 3.4 (m, 1H, C12), 4.95(m, 2H, C21), 5.91(s, 1H, C22). M.S. : (EI) 473 M+

**d. Boc-linked dig 10**




10c

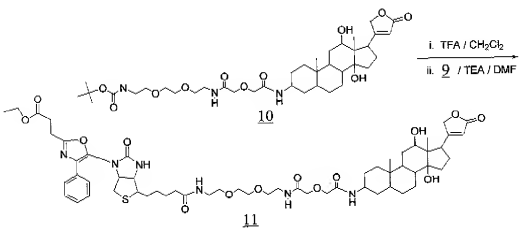



10d

To a solution of N-tboc acid **10c** ( Aldrich, 721 mg, 2.0 mmol) in THF (25 ml) were added DCC (412 mg, 2.0 mmol) and NHS (287 mg, 2.5 mmol). The mixture was stirred

overnight. The reaction was then filtered through glass wool and added to a stirred solution of aminodigoxigenin **10c** and triethyl amin (101 mg) in 25 ml of dichloromethane. The reaction was stirred overnight and then was concentrated under vacuum . Dichloromethane (50 ml) was then added and extracted with 3x50 ml of 0.5 N HCl and finally with water (100ml). The organic phase was then dried and evaporated to 1/3 of the volume and applied to ten 20x20 silicagel GF plates ($CH_3OH$:$H_2O$, 1:9 ), to give 1.2 g of the pure product.

**5. Preparation of Digoxigenin-Linked-Biotin-Oxazole-R-$CO_2$Et (11).**



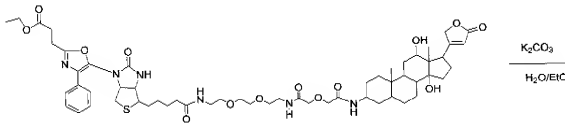

10

11

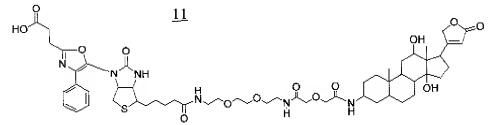
Boc-Linked-Dig (**10**) (30 mg, 0.04 mmol) was deprotected by stirring in 1.0 ml (1:5) TFA-$CH_2Cl_2$ for 15 min at ambient temperature. The reaction mixture was concentrated and the volatiles removed by dissolving the residue in $CH_2Cl_2$ and concentrating a second time. The residue was redissolved in 2 mL $CH_2Cl_2$ and neutralized with TEA in 1 mL DMF. The NHS-Biotin-Oxazole-R-$CO_2$Et filtrate (**9**) was rinsed with 0.5 mL THF directly into the DMF diluted amino-Linked-Dig solution. The solution was bubbled with argon and stirred for 2h at ambient temperature. TLC ($SiO_2$ : 10% MeOH/$CH_2Cl_2$) confirmed that the reaction had gone to completion. The crude reaction mixture was applied directly to a pre-equilibrated (5% MeOH/$CH_2Cl_2$) preparative silica gel plate and eluted with 15% MeOH/$CH_2Cl_2$. The pure product fractions were combined affording 16 mg DLBOR-$CO_2$Et (**11**). TLC ($SiO_2$: 0.1% HOAc, 15% MeOH/$CH_2Cl_2$, single spot $R_f$ 0.3). 1H-

NMR (250MHz, $D_2O$) δ: 7.9 – 7.1 (m, 5H), 5.7 (s, 1H), 5.65 (s, 1H), 4.9 (s, 2H), 4.6 (7H + $H_2O$), 4.25 (m, 2H), 4.1 (m, 2H), 3.95 (m, 2H), 3.65 (m 2H), 3.6 – 3.25 (m, 10H), 3.25 – 2.85 (m, 7H), 2.85 (m, 2H), 2.25 (M, 1H), 2.0 (m, 2H), 1.75 (m, 2H), 1.6 – 0.9 (m, 26H), 0.75 (s, 3H), 0.60 (s, 3H).

**6. Preparation of Dig-Linked-Biotin-Oxazole-R acid (DLBOR-$CO_2$H) (12).**




11

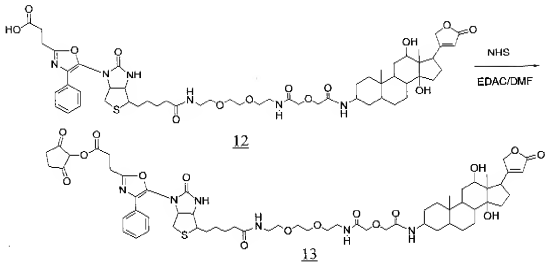


12

To 10.0 mg DLBOR-$CO_2$Et (**11**) (9.0 umol), dissolved in 100 uL EtOH, was added 1.0 mL of 25 mM $K_2CO_3$ in 5% $H_2O$/EtOH. The solution was stirred under argon overnight at ambient temperature. The reaction was followed by TLC ($SiO_2$ : 5% MeOH/$CH_2Cl_2$), after 18h the pH of the reaction was adjusted to 3-4 with 0.1 N HCl. TLC (C18 : 95% $CH_3CN/H_2O$, major spot $R_f$ 0.5, and $SiO_2$ : 0.1% HOAc, 15% MeOH/$CH_2Cl_2$, major spot $R_f$ 0.65, and a minor spot $R_f$ 0.90 (the minor spot appeared to be digoxin-linked biotin), NMR confirmed the complete hydrolysis of the ethyl ester. Dig-Linked-Biotin (DLB) release by $^1O_2$ was confirmed by the soluble sensitizer method (see Example 5). The solvent was removed under vacuum and a 2.0 mg (1.7 umol) portion of the crude DLBOR acid (**12**) was redissolved in 0.50 mL DMF for use in the next step.

**7. Preparation of Dig-Linked-Biotin-Oxazole-R-NHS ester (DLBOR-$CO_2$NHS**

**ester) (13).**




12

13

To 2.0 mg (1.7 umol) DLBOR-$CO_2$H (**12**) in 0.5 mL DMF was added 10 uL of NHS (0.23 mg, 2.0 umol)/DMF and EDAC. The solution was stirred under argon overnight at ambient temperature. The solvent was removed under vacuum and the residue was redissolved in 100 uL of degassed DMF. This DMF solution of DLBOR-$CO_2$NHS (**13**) was attached directly to beads in the same manner as described for DLBAR-$CO_2$NHS (see **Figure 3** and Example 2).